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(54) Title: *IN VITRO* METHODS FOR IDENTIFYING MODULATORS OF MEMBERS OF THE STEROID/THYROID SUPERFAMILY OF RECEPTORS

(57) Abstract

In accordance with the present invention, there are provided *in vitro* methods for the large scale identification of modulators of members of the steroid/thyroid superfamily of receptors. Invention methods can be rapidly carried out employing large numbers of compounds per screen, thereby facilitating evaluation of large libraries of compounds in a relatively short time. Modulators identified employing invention methods are capable of activating a receptor species in the optional presence of one or more of: a heterodimerizing partner therefor, a co-activator (or co-suppressor), and/or a hormone response element; or of disrupting the activity of a receptor species in the presence or absence of one or more of: a heterodimerizing partner therefor, a co-activator (or co-suppressor), and/or a hormone response element.

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In Vitro Methods for Identifying Modulators of Members of the Steroid/Thyroid Superfamily of Receptors

FIELD OF THE INVENTION

The present invention relates to intracellular receptors, and modulators therefor. Broadly, the present invention relates to methods for the identification of compounds which function as modulators for intracellular receptors. In a particular aspect, the present invention relates to methods for the identification of compounds which activate intracellular receptors. In another particular aspect, the present invention relates to methods for the identification of compounds which suppress activation of intracellular receptors.

BACKGROUND OF THE INVENTION

A central problem in eukaryotic molecular biology continues to be the elucidation of molecules and mechanisms that mediate specific gene regulation. As part of the scientific attack on this problem, a great deal of work has been done in efforts to identify modulators (i.e., exogenous inducers or repressors) which are capable of mediating specific gene regulation.

Although much remains to be learned about the specifics of gene regulation, it is known that gene transcription is modulated by the interaction of intracellular components, including intracellular receptors and discrete DNA sequences known as hormone response elements (HREs) with modulators.

As additional members of the steroid/thyroid superfamily of receptors are identified, the identification of ex genous inducers (i.e., naturally occurring (or synthetic) inducers) and/or exogenous suppressors (i.e., naturally occurring (or synthetic) suppressors) for such

newly discover d receptors is highly desirable. Indeed, the identification of compounds which directly or indirectly interact with intracellular receptors, and thereby affect transcription of hormone-responsive genes, would be of significant value, e.g., for therapeutic applications. Frequently, however, modulators for these novel receptors can not readily be identified. Accordingly, methods for the ready identification of modulators for such receptors would be of great value. Of particular value would be in vitro methods which can be carried out on large scale, thereby facilitating the screening of large numbers of compounds.

It has recently been discovered that some intracellular receptors function to regulate transcription only when associated with additional transcriptionally active components, such as another member of the steroid/thyroid superfamily of receptors (i.e., as a heteromer, typically a heterodimer). For those receptors which typically function as part of a heterodimer, the availability of compounds which are modulators of heterodimer formation and/or activity are of great interest. Thus, for example, it would be desirable to be able to identify compounds which are capable of activating (or repressing activation of) said receptor in the absence of the heterodimerizing partner therefor; or compounds which are capable of activating (or repressing activation of) said receptor only in the presence of its heterodimerizing partner.

Other information helpful in the understanding and practice of the present invention can be found in commonly assigned United States Patent Nos. 4,981,784, 5,071,773, 5,091,518 and 5,260,432; and United States Pat nt Application No. 325,240, filed March 17, 1989, now aband n d, and CIP th reof, United Stat s Patent Application No. 494,618, filed March 16, 1990, now pending;

all of which are hereby incorporated herein by reference in their entirety.

BRIEF DESCRIPTION OF THE INVENTION

In accordance with the present invention, we have developed *in vitro* methods for the large scale identification of modulators of members of the steroid/thyroid superfamily of receptors. Invention methods can be rapidly carried out employing large numbers of compounds per screen, thereby facilitating evaluation of large libraries of compounds in a relatively short time.

Modulators identified employing invention methods are capable of activating a receptor species in the presence of a heterodimerizing partner therefor, or of disrupting the activity of a receptor species in the presence or absence of a heterodimerizing partner therefor.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there are provided methods for the large scale identification of modulators for a single member of the steroid/thyroid superfamily of receptors, said method comprising:

individually contacting each of a plurality of test compounds with said member, wherein said member is optionally associated with one or more of a heteromeric partner therefor, a co-activator (or co-suppressor), and/or a hormone response element,

assaying for the formation or disruption of a complex comprising at least said test compound and said member, and

identifying as modulators those compounds which participate in the formation or disruption of said complex.

In accordance with another mbodiment of the present invention, there are provid d methods for the large scale screening of a collection of members of the steroid/thyroid superfamily of receptors to identify those member(s) of said collection for which a given test compound may serve as a modulator, said method comprising:

individually contacting each of a plurality of said members with a test compound, wherein said members are optionally associated with one or more of a heteromeric partner therefor, a co-activator (or co-suppressor), and/or a hormone response element,

assaying for the formation or disruption of a complex comprising at least said test compound and said member, and

identifying as modulators those compounds which participate in the formation or disruption of said complex.

Any member of the steroid/thyroid superfamily of receptors can be used in the assays of the invention. As employed herein, the phrase "members of the steroid/thyroid superfamily of receptors" (also known as "nuclear receptors" or "intracellular receptors") refers to hormone binding proteins that operate as ligand-dependent transcription factors, including identified members of the steroid/thyroid superfamily of receptors for which specific modulators have not yet been identified (referred to hereinafter as "orphan receptors"). These hormone binding proteins have the intrinsic ability to bind to specific DNA sequences. Following binding, the transcriptional activity of target gene (i.e., a gene associated with the specific DNA sequence) is modulated as a function of the compound bound to the receptor.

The DNA-binding domains of all of these nucl ar receptors are r lated, consisting of 66-68 amino acid

residues, and possessing about 20 invariant amino acid residues, including nine cysteines.

A member of the superfamily can be identified as a protein which contains the above-mentioned invariant amino acid residues, which are part of the DNA-binding domain of such known steroid receptors as the human glucocorticoid receptor (amino acids 421-486), the estrogen receptor (amino acids 185-250), the mineralocorticoid receptor (amino acids 603-668) and the human retinoic acid receptor (amino acids 88-153). The highly conserved amino acids of the DNA-binding domain of members of the superfamily are as follows:

Cys - X - X - Cys - X - X - Asp* - X -
Ala* - X - Gly* - X - Tyr* - X - X -
X - X - Cys - X - X - Cys - Lys* -
X - Phe - Phe - X - Arg* - X - X - X -
X - X - X - X - X - X - (X - X -) Cys -
X - X - X - X - X - (X - X - X -) Cys -
X - X - X - Lys - X - X - Arg - X - X -
Cys - X - X - Cys - Arg* - X - X -
Lys* - Cys - X - X - X - Gly* - Met
(SEQ ID No 1);

wherein X designates non-conserved amino acids within the DNA-binding domain; the amino acid residues denoted with an asterisk are residues that are almost universally conserved, but for which variations have been found in some identified hormone receptors; and the residues enclosed in parenthesis are optional residues (thus, the DNA-binding domain is a minimum of 66 amino acids in length, but can contain several additional residues).

Exemplary members of the steroid/thyroid superfamily of receptors include steroid receptors such as glucocorticoid receptor (GR), mineralocorticoid receptor

(MR), progestrone receptor (PR), androgen receptor (AR), vitamin D_3 receptor (VDR), and the like; plus retinoid receptors, such as various isoforms of the retinoic acid receptor (e.g., RARα, RARβ, RARγ, and the like), plus various isoforms of the retinoid X receptor (e.g., RXRα, RXRβ, RXRγ, and the like); various isoforms of the thyroid hormone receptor (e.g., TRα, TRβ, and the like); as well as other gene products which, by their structure and properties, are considered to be members of the superfamily, as defined hereinabove.

Examples of orphan receptors include various isoforms of HNF4 (see, for example, Sladek et al., in Genes & Development 4:2353-2365 (1990)), the COUP family of receptors (e.g., COUPα or COUPβ; see, for example, Miyajima et al., in Nucleic Acids Research 16:11057-11074 (1988), Wang et al., in Nature 340:163-166 (1989), including the COUP-like receptors and COUP homologs, such as those described by Mlodzik et al., in Cell 60:211-224 (1990) and Ladias et al., in Science 251:561-565 (1991)), ultraspiracle (see, for example, Oro et al., in Nature 347:298-301 (1990)), various isoforms of peroxisome proliferator activated receptor (e.g., PPARα, PPARγ or PPARδ; see, for example, Dreyer et al., in Cell 68:879-887 (1992)), orphan receptor XR2 and various isoforms thereof (e.g., XR2α; see, for example, United States Patent Application Serial No. 07/761,068, now pending), constitutively active receptor, CAR, and various isoforms thereof (also known as "MB67"; see, for example, Baes et al., in Mol. Cell. Biol. 14:1544-1552 (1994)), various isoforms of the orphan receptor NGFI-B (e.g., NGFI-Bα or NGFI-Bβ; see, for example, Milbrandt in Neuron 1:183-188 (1988) and Scearce et al., in J. Biol. Chem. 268:8855-8861 (1993)), various isoforms of the liver-derived receptor referred to as LXR (see, for example, United States Application Serial No. 08/373,935, filed January 13, 1995, now pending, as well as the receptor described by Apfel et

al., in Mol. Cell. Biol. 14:7025-7035 (1994) and the receptor described by Song et al., in Proc. Natl. Acad. Sci. USA 91:10809-10813 (1994)), various isoforms of the farnesoid X receptor (FXR; see, for example, Forman et al., in Cell 81:687-693 (1995)), and the like.

Presently preferred members of the steroid/thyroid superfamily of receptors contemplated for use in the practice of the present invention are selected from various isoforms of PPAR, VDR, CAR, LXR, FXR, NGFI-B, and the like.

As employed herein, the term "modulator" refers to a wide range of compounds and/or conditions which can, either directly or indirectly, exert an influence on the activation and/or repression of the receptor of interest (optionally associated with one or more of a heterodimerizing partner therefor, a co-activator and/or a hormone response element). Thus, a ligand precursor (i.e., a compound that can be converted into a ligand) is a modulator. Similarly, a compound which converts a ligand precursor into an active ligand is also a modulator. Furthermore, the precursor of a modulator (i.e., a compound that can be converted into a modulator) is also considered to be a modulator. Similarly, a compound which converts a precursor into a modulator is also considered to be a modulator.

As readily recognized by those of skill in the art, a wide variety of test compounds can be employed in the invention assays. Examples of the classes of compounds contemplated for use in the practice of the present invention include steroids, retinoids, prostaglandins, leukotrienes, thiazolidinediones, farnesoids, aminobenzoates, hydroxybenzoates, eicosanoids, cholesterol metabolites, fibrates, amino acids, sugars, nucleotides, fatty acids, lipids, serotonin, dopamine, catecholamines,

acid azol s, and the lik . In a particular asp ct, th plurality of test compounds employed in the inv ntion assays can comprise a combinatorial library, wherein each individual test compound is one of an array of structurally related compounds.

As readily recognized by those of skill in the art, contacting contemplated by the above-described assays can be carried out in solution, or in the solid phase. Where assays are conducted in solution, all components are dissolved or suspended in suitable media. The formation or disruption of complex caused by the presence of test compound can then be readily assayed in a variety of ways, as described in greater detail hereinbelow.

Where assays are conducted in solid phase, one or more of the components of the assay are immobilized on a suitable support, which is then exposed to the other components of the assay. The formation or disruption of complex caused by the presence of test compound can then be readily assayed in a variety of ways, as described in greater detail hereinbelow.

As noted previously, contacting contemplated by the invention assays can optionally be carried out in the presence of a heteromeric partner for the member of the superfamily, whereby the complex induced by the presence of test compound (or the complex disrupted by the presence of test compound) comprises said member and heteromeric partner therefor, optionally further containing a hormone response element and/or co-activator (or co-suppressor) therefor. The presently preferred heteromeric partner contemplated for use in the practice of the present invention is RXR.

Alternatively, contacting contemplated by the invention assays can optionally be carried out in the

presence f a co-activator (or co-suppressor) for the member of the superfamily, whereby the complex induced by the presence of test compound (or the complex disrupted by the presence of test compound) comprises said member and said co-activator (or co-suppressor), optionally further containing a heteromeric partner and/or hormone response element therefor.

Co-activators (and co-suppressors) contemplated for use in the practice of the present invention include SRC-1 (see, for example, Onate et al., in Science 270:1354-1357 (1995)), Tif (see, for example, LeDouarin et al., in EMBO Journal 14:2020-2033 (1995) and Baur et al., in EMBO Journal 15:110-124 (1996)), trip (see, for example, Lee et al., in Nature 374:91-94 (1995)), Rip_{140} (see, for example, Cavailles et al., in EMBO Journal 14:3741-3751 (1995)), ERAP (see, for example, Halachmi et al., in Science 264:1455-1458 (1994)), N-CoR (see, for example, Kurokawa et al., in Nature 377:451-454 (1995)), and the like.

As yet another alternative, contacting contemplated by the invention assays can optionally be carried out in the presence of a hormone response element for the member of the superfamily, whereby the complex induced by the presence of test compound (or the complex disrupted by the presence of test compound) comprises said member and said hormone response element, optionally further containing a heteromeric partner and/or co-activator (or co-suppressor) therefor.

Hormone response elements contemplated for use in the practice of the present invention are well known and have been thoroughly described in the art. Such response el ments can include dir ct rep at structures or inverted r peat structur s based on well defined hexad half sites, as described in gr ater detail below. Exemplary hormone

r sponse lem nts are composed of at least on direct repeat of two or mor half sit s, separated by a spac r having in the range of 0 up to 6 nucleotides. The spacer nucleotides can be randomly selected from any one of A, C, G or T. Each half site of response elements contemplated for use in the practice of the invention comprises the sequence:

-RGBNNM-,

wherein

R is selected from A or G;

B is selected from G, C, or T;

each N is independently selected from A, T, C, or G; and

M is selected from A or C;

with the proviso that at least 4 nucleotides of said -RGBNNM- sequence are identical with the nucleotides at corresponding positions of the sequence -AGGTCA-. Response elements employed in the practice of the present invention can optionally be preceded by N_x, wherein x falls in the range of 0 up to 5.

Those of skill in the art recognize that assays to determine the formation or disruption of receptor-containing complexes contemplated by the invention method can be carried out in a wide variety of ways. Example methods include gel shift assays (see, for example, Forman et al., in Cell 81:687-693 (1995)), immunological/affinity methods (see, for example, Yao et al., in Nature 366:476-479 (1993)), surface plasmon resonance (see, for example, Fisher and Fivash in Curr. Opin. Biotechnol. 5:389-395 (1994)), circular dichroism and optical rotary dispersion (see, for example, Toney et al., in Biochemistry 32:2-6 (1993)), fluorescence anisotropy (see, for example, Kersten t al., in Biochemistry 34:13717-13721 (1995)), nuclear magnetic resonanc (see, for example, Jenkins in Lif Sciences 48:1227-1240 (1991)), and the like.

As noted previously, invention methods are amenable to being conducted on a large-scale. For example, each of the steps contemplated herein (i.e., contacting, assaying and identifying) can be substantially simultaneously carried out employing a format suitable for multiple exposures at the same time, e.g., employing a multi-well plate. The large scale screening contemplated by the invention can be rendered even more time effective by automating the process. For example, a plurality of test compounds (e.g., various members of a combinatorial library) can be placed into individual wells of a 96-well plate, wherein each well contains (or subsequently has added thereto) a receptor of interest, optionally in the presence of heteromeric partner therefor, a co-activator (or co-suppressor) and/or a hormone response element; each well can then be assayed to determine whether formation or disruption of complex is induced by test compound; and those compounds having such effect readily identified and selected for further testing as appropriate. Employing such a format, it can readily be seen that numerous test compounds can be assayed over relatively short periods of time.

The invention will now be described in greater detail by reference to the following non-limiting example.

Example 1
Gel Shift Protocol

Electrophoretic mobility shift assays were performed using proteins translated in a rabbit reticulocyte lysate system (TNT, Promega). Proteins (0.1-1 µl) were incubated with or without a specific modulator/ligand for 30 minutes at room temperature with 100,000 cpm of Klenow-labeled prob sin 10 mM Tris pH 8, 150 mM KCl, 6% glycerol, 0.05% NP-40, 1 mM DTT, 100ng/µl poly dI·dC and then electrophoresed through a 5% polyacrylamide

gel in 0.5x TBE (45 mM Tris•base, 45 mM boric acid and 1 mM of EDTA. See Forman et al., in Cell 81:687-693 (1995).

While the invention has been described in detail with reference to certain preferred embodiments thereof, it will be understood that modifications and variations are within the spirit and scope of that which is described and claimed.

SEQUENCE LISTING

SEQ ID NO:1

Cys - X - X - Cys - X - X - Asp* - X -
Ala* - X - Gly* - X - Tyr* - X - X -
X - X - Cys - X - X - Cys - Lys* -
X - Phe - Phe - X - Arg* - X - X - X -
X - X - X - X - X - X - (X - X -) Cys -
X - X - X - X - X - (X - X - X -) Cys -
X - X - X - Lys - X - X - Arg - X - X -
Cys - X - X - Cys - Arg* - X - X -
Lys* - Cys - X - X - X - Gly* - Met

That which is claimed is:

1. A method for the large scale identification of modulators for a member of the steroid/thyroid superfamily of receptors, said method comprising:

individually contacting each of a plurality of test compounds with said member, wherein said member is optionally associated with one or more of a heteromeric partner therefor, a co-activator (or co-suppressor), and/or a hormone response element,

assaying for the formation or disruption of a complex comprising at least said test compound and said member, and

identifying as modulators those compounds which participate in the formation or disruption of said complex.

2. A method according to claim 1 wherein said contacting is carried out in the presence of a heteromeric partner for said member.

3. A method according to claim 1 wherein said complex comprises said member and heteromeric partner therefor.

4. A method according to claim 3 wherein said complex further comprises a co-activator (or co-suppressor).

5. A method according to claim 3 wherein said complex further comprises a hormone response element.

6. A method according to claim 1 wherein said complex comprises said member and a co-activator (or co-suppr ssor).

7. A method according to claim 1 wherein said complex comprises said member and a hormone response element.

8. A method according to claim 1 wherein said member of the steroid/thyroid superfamily of receptors forms a complex with heteromeric partner therefor in the presence of ligand therefor.

9. A method according to claim 1 wherein said member of the steroid/thyroid superfamily of receptors forms a complex with a co-activator (or co-suppressor) in the presence of ligand therefor.

10. A method according to claim 9 wherein said co-activator (or co-suppressor) is selected from SRC-1, Tif, trip, Rip_{140}, ERAP or N-CoR.

11. A method according to claim 1 wherein said compound disrupts complex comprising said member of the steroid/thyroid superfamily of receptors.

12. A method according to claim 1 wherein said test compound is selected from steroids, retinoids, prostaglandins, leukotrienes, thiazolidinediones, farnesoids, aminobenzoates, hydroxybenzoates, eicosanoids, cholesterol metabolites, fibrates, amino acids, sugars, nucleotides, fatty acids, lipids, serotonin, dopamine, catecholamines or acid azoles.

13. A method according to claim 12 wherein said test compound is part of a combinatorial library.

14. A method according to claim 1 wherein said contacting is carried out in solution.

15. A method according to claim 1 wherein said contacting is carried out in solid phas .

16. A method according to claim 1 wherein said heteromeric partner is RXR.

17. A method according to claim 1 wherein said co-activator (or co-suppressor) is selected from SRC-1, Tif, trip, Rip_{140}, ERAP or N-CoR.

18. A method according to claim 1 wherein a plurality of said contacting, assaying and identifying steps are substantially simultaneously carried out in a multi-well plate.

19. A method according to claim 18 wherein said plurality of contacting, assaying and identifying steps are automated.

20. A method for the large scale identification of a modulator for members of the steroid/thyroid superfamily of receptors, said method comprising:

individually contacting each of a plurality of said members with a test compound, wherein said members are optionally associated with one or more of a heteromeric partner therefor, a co-activator, and/or a hormone response element,

assaying for the formation or disruption of a complex comprising at least said test compound and said member, and

identifying as modulators those compounds which participate in the formation or disruption of said complex.

21. A method according to claim 20 wherein said contacting is carried out in the presence of a heterom ric partn r for each of said members.

22. A method according to claim 20 wherein said complex comprises ne of said members and heteromeric partner therefor.

23. A method according to claim 22 wherein said complex further comprises a co-activator (or co-repressor).

24. A method according to claim 22 wherein said complex further comprises a hormone response element.

25. A method according to claim 20 wherein said complex comprises one of said members and a co-activator (or co-suppressor).

26. A method according to claim 20 wherein said complex comprises one of said members and a hormone response element.

27. A method according to claim 20 wherein each of said members of the steroid/thyroid superfamily of receptors forms a complex with heteromeric partner therefor in the presence of ligand therefor.

28. A method according to claim 20 wherein each of said member of the steroid/thyroid superfamily of receptors forms a complex with a co-activator (or co-suppressor) in the presence of ligand therefor.

29. A method according to claim 28 wherein said co-activator (or co-suppressor) is selected from SRC-1, Tif, trip, Rip_{140}, ERAP or N-CoR.

30. A method according to claim 20 wherein said compound disrupts complex comprising said member of the steroid/thyroid superfamily of receptors.

31. A method according to claim 20 wherein said test compound is selected from st roids, retinoids, prostaglandins, leukotrienes, thiazolidinediones, farnesoids, aminobenzoates, hydroxybenzoates, eicosanoids, cholesterol metabolites, fibrates, amino acids, sugars, nucleotides, fatty acids, lipids, serotonin, dopamine, catecholamines or acid azoles.

32. A method according to claim 20 wherein said contacting is carried out in solution.

33. A method according to claim 20 wherein said contacting is carried out in solid phase.

34. A method according to claim 20 wherein said co-activator (or co-suppressor) is selected from SRC-1, Tif, trip, Rip_{140}, ERAP or N-CoR.

35. A method according to claim 20 wherein a plurality of said contacting, assaying and identifying steps are substantially simultaneously carried out in a multi-well plate.

36. A method according to claim 35 wherein said plurality of contacting, assaying and identifying steps are automated.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/04315

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :G01N 33/53; C12Q 1/02
US CL :435/7.1, 29
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/7.1, 29

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, EMBASE, BIOSIS, CAPLUS
search terms: screen, bioassay etc., steroid/thyroid family receptors, src-1 and synonyms

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P ---- Y, P	PETTY et al. A TATA binding protein-associated factor functions as a co-activator for thyroid receptors. Mol. Endocrin. December 1996, Vol. 10, No. 12, pages 1632-1645, especially pages 1633-1643.	1-9, 11-16, 18-19 ----- 13,15, 18-19
X, P ---- Y, P	TAKASHITA et al. Molecular cloning and properties of a full-length putative thyroid hormone receptor coactivator. Endocrin. August 1996, Vol. 137, No. 8, pages 3594-3597, especially pages 3594-3596.	1-12, 14, 16-17 ----- 13, 15, 18-19
X ---- Y	PERLMAN et al. A novel pathway for vitamin A signalling mediated by RXR heterodimerization with NGFI-B and NURR-1. Genes & Development. 1995, Vol. 9, pages 769-782, especially pages 770-778.	1-9, 11-16, 18-19 ------ 13,15, 18-19

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
06 MAY 1997

Date of mailing of the international search report
30 JUL 1997

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/04315

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P ---- Y, P	ZHU et al. Cloning and identification of mouse steroid receptor coactivator-1 (mSRC-1), as a coactivator of peroxisome proliferator-activated receptor-gamma. Gene Expression. December 1996, Vol. 6, No. 3, pages 185-195, especially pages 186-192..	1-12, 14, 16-17 ----- 13,15, 18-19

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/04315

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-19, species A

Remark on Pr test

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/04315

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1-19, drawn to a method for the large scale identification of multiple modulators for a single member of the steroid/thyroid superfamily of receptors.

Group II, claim(s) 20-36, drawn to a method for the large scale identification of a single modulator for multiple members of the steroid/thyroid superfamily of receptors.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be searched, the appropriate additional search fees must be paid. The species are as follows:

A) co-activator (or co-suppressor): SRC-1

B) co-activator (or co-suppressor): TIF

C) co-activator (or co-suppressor): trip

D) co-activator (or co-suppressor): RIP_{140}

E) co-activator (or co-suppressor): ERAP

F) co-activator (or co-suppressor): N-COR

The claims are deemed to correspond to the species listed above in the following manner:

A-F) in claims 10 and 17 of Group I

F) in claims 29 and 34 of Group II

The following claims are generic: claims 1 and 20

The inventions listed as Groups I and II do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The method of identifying multiple modulators for a single member of the steroid/thyroid superfamily of receptors of Group I, and the method of identifying a single modulator for multiple members of the steroid/thyroid superfamily of receptors of Group II, each have materially different chemical and functional properties, require functionally and chemically different process steps, and achieve materially, functionally and chemically distinct ends. These chemical and functional properties/process steps are the special technical feature that identify each invention and distinguish each invention from the others and because the aforementioned groups require chemically and functionally distinct process steps as their special technical feature, no special technical feature is shared by the separate groups.

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: The co-activators (or co-suppressors) of species A-F (i.e. SRC-1, TIF, trip, RIP_{140}, ERAP and N-COR respectively) each have materially different chemical structures and materially different functional properties. These chemical structures and functional properties are the special technical features that identify each individual species and distinguish each species from the others, because none of the special technical features is shared by the species listed above.